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14. ABSTRACT

Maintaining genomic stability is critical for organismal fitness. Consequently, the absence of tumor suppressor gene activity, such as p53, results in increased genomic instability and increased cancer predisposition. Homologous recombination (HR), as measured by the in vivo pun assay, is a DNA repair mechanism that our laboratory uses to measure genomic instability. We compared eyespot frequency in normal wild type mice, mice that are absent in p53 protein (null) and those that have the hotspot mutations R172H and R172P (equivalent to R175 in human breast cancer). Previously, we have shown that in the absence of p53 the normal frequency of spontaneous HR is significantly elevated. However, the mechanism by which p53 suppresses HR is unclear. The p53 R172P mutant mice retains limited transcriptional functionality (regulating cell cycle genes but not apoptotic genes) while the p53 R172H mutant mice lack any transcriptional activity but retain some protein: protein interaction capability. We observed significantly increased HR frequency in the p53 R172H mutant versus the p53 R172P mutant mice. This suggests that p53 regulation of cell cycle genes but not apoptotic genes may be responsible for its ability to suppress HR frequency. Also, the loss of key protein: protein interactions may have contributed to this suppression. It has been previously reported that p53 R172H mutant mice come down with early aggressive tumors compared to the p53 R172P mutant mice. This correlates with the increased HR frequency we observed in the R172H mutant mice implicating p53 suppression of genomic instability as a major mechanism for p53 tumor suppression. This work provides novel insight into the mechanism of cancer development in the absence or mutation of p53 and the mechanism of p53 control of HR in an in vivo system. p53 is often a targeted therapy and further insight into the function of p53 in DNA repair pathways can be vital to finding novel points of targeted therapy. Our data will add insight to the important paradigm of genomic instability and its relation to breast cancer etiology.

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Table of Contents

Introduction

The purpose of this project is to determine the mechanism for how the tumor suppressor, p53, suppresses homologous recombination. P53 is implicated in 50% of all human cancers and inactivated in some form in 100% of human cancers. Homologous recombination (HR) is an error proof repair mechanism that is able to repair any type of DNA lesion with high fidelity. However, when the HR machinery uses an incorrect template for repair large deletions in the genome can occur leading to a predisposition for cancer. P53 has been implicated in suppressing homologous recombination in order to maintain genomic stability, however the mechanism is still unknown. In the first year of this grant huge strides have been made in the numbers of mice breed and relevant cells collected for the purposes of experiments outlined in the aims below. The PI has optimized the pun assay and mouse husbandry in the first year of this grant and has collected data during the second year of the grant period. The second year accomplishments include three middle author publications due to the PI's knowledge, and expertise in various areas including p53 mutation, DNA repair and cell cycle function. This expertise will help the PI publish work for the aims outlined below. The PI has also attended two conferences this year where she presented a poster and has had several committee meetings to evaluate the work progress. Experiments are being completed in order to submit a first author manuscript in March of 2012 for work completed in accordance with this grant.

Body

P53 is a potent tumor suppressor that shields the genome from daily interrogations of endogenous and exogenous damage, most importantly through its ability to arrest the cell cycle. In response to damage, p53 up regulates transcription of p21 leading to G1 arrest, which allows adequate time for repair of lesions before entering S phase (1, 2). Furthermore, p53 has been linked to G2/M arrest through multiple overlapping p53-dependent and p53-independent pathways that inhibit cdc2 (3). As a final resort if the damage is severe enough p53 has been shown to induce apoptosis in certain situations (2, 4).

P53 has also been linked to various DNA repair pathways such as non-homologous end joining (NHEJ) and homologous recombination (HR). Homologous recombination is a high fidelity DNA repair mechanism that can repair almost any type of DNA lesion when in correct equilibrium. When this delicate balance is disrupted as seen in *Blm* null cells resulting in hyperrecombination or hyporecombination in *Brca1* null cells the ensuing result is genomic instability (5).

It has been reported previously that p53 down regulates spontaneous homologous recombination in chromosomally integrating plasmid substrate models. Bertrand *et al*. using a plasmid-based system with PJS3-10 (mouse L cell lines) overexpressed the mutant $p53^{175\ (Arg>His)}$, which showed a 5-20 fold increase in spontaneous recombination compared to wild type control cells. Further analysis showed that the effect of the p53 mutation acted on both rad51 dependent gene conversion events and deletion events (6).

Willers *et al*. also showed an increase in recombination frequency in a temperature sensitive p53 mutant (Ala135 to Val) using a plasmid substrate that stably integrated into p53 null mouse embryonic fibroblasts (MEFs). This study further established the uncoupling of p53's function in suppressing HR and its role as a cell cycle checkpoint protein (7).

The Wiesmuller lab has explored the role of p53 in HR using a rare cutting endonuclease ISCE-1 in breast cancer cells with varying p53 mutations. This study used a DSB repair assay to show that some p53 mutants retain partial ability to repair double strand breaks by repressing aberrant HR and less infrequently through NHEJ and SSA(8).

P53 is mutated in 50% of all human cancers and most likely inactivated by some other mechanism in the other 50%. Patients with Li Fraumeni syndrome suffer from a germ line mutation in p53 and subsequently endure an early onset of cancer. Mouse models have been created to recapitulate this phenomenon and are surprisingly viable. 80% of P53 null mice come down with lymphomas within 6 months and the rest suffer from sarcomas. MEFs from these mice show aneuploidy, allelic loss and gene amplification. Most of these germline mutations are missense mutations occurring in the DNA binding domain of p53. One such mutant is the p53-R172P and p53-

R172H mouse model (9). The p53-R172P mouse is able to induce partial cell cycle arrest in response to DNA damage but is defective in promoting apoptosis. Mice homozygous for this mutation escape the early onset of lymphomas that is typical for p53 null mice, however these mice eventually do succumb to tumors that have a normal diploid number of chromosomes in contrast to p53 null tumors. The p53-R172H mouse shows an inability to transactivate p53 target genes as well as a defect in apoptosis induction (10). A majority of mice homozygous for p53-R172H developed lymphomas similar to p53 null mice with a smaller percent developing sarcomas. P53-R172H heterozygous mice developed sarcomas and a surprising number of osteosarcomas and carcinomas that metastasized, which was not seen in p53 heterozygous mice (9). Interestingly, the p53-R172H tumors showed a high level of aneuploidy similar to p53 null mice but unlike p53-R172P mice. Given this we sought to look at the HR frequency of these two mutants to determine if there is a difference in in the ability to suppress HR similar to WT given the different functionalities of these to mutants. HR is measure of genomic instability, even though it can fix any type of genotoxic lesion, when used incorrectly it can cause large deletions and lesions in the genome. Using the in vivo pun assay we have seen an increase in HR frequency in many mouse models of the DNA damage repair pathway. HR frequency is increased in BLM null, p53 null and parp null mice and decreased in Brca1 and Brca2 null animals (5, and unpublished work).

Given the power of this assay here we used the *in vivo* p^{un} assay to determine the consequence of HR suppression in two breast cancer hotspot p53 mutant mouse models with differing loss of function. The p53-R172P mice, which are defective in their ability to induce apoptosis but are able to induce cell cycle genes, retained the ability to suppress HR similar to wild type p53 animals. The more aggressive p53-R172H mouse showed increase HR similar to p53 null mice, which do not produce any p53 protein at all.

Specific Aim 1: Determine whether p53 mutants R172P and R172H suppress spontaneous levels of homologous recombination the same as wild-type p53.

In the first year of the training grant great effort was put forth to establish a robust breeding colony of R172P, R172H, Wild type and p53 null mice in order to have sufficient numbers of animals to perform the in vivo pun assay in the second year of training.

p^{un} Eyespot HR Assay

Heterozygous mice from the breeding cohorts established in the first year: $p53^{R172P/+}$ $p^{un/un}$, $p53^{R172H/+}$ $p^{un/un}$ and $p53^{neo/+}$ $p^{un/un}$ mice were intercrossed in each respective cohort to produce the desired experimental mice ($p53^{R172P/R172P}$, $p53^{R172H/R172H}$, $p53^{neo/neo}$) along with littermate controls ($p53^{R172P/+}$,

p53$^{R172H/+}$, *p53*$^{neo/+}$, *p53*$^{+/+}$). Mice were then sacrificed at weaning age and their eyes harvested and dissected to expose the retinal pigment epithelium (RPE) as previously described (12). Briefly, each RPE whole mount was digitally photographed and analyzed for eyespots using a Zeiss Lumar version 12 stereomicroscope, Zeiss AxioVision MRm camera, and Zeiss AxioVision 4.6 software (Thornwood, NY) as described previously (13, appendix). The criteria for what constitutes an eyespot was previously defined in bishop *et al*. as being a pigmented cell that is separated by 2 other pigmented cells (12). Next, the RPE images were uploaded into Adobe Photoshop and the edge of the RPE was delineated using the ellipse tool and free transform path function. Two measurements were made (i) a frequency of eyespots (HR events) per RPE, and (ii) distribution of the eyespots within the RPE (their position) reflecting the developmental time at which the eyespots were produced.

Statistics

The Kruskal-Wallis test (non-parametric, one-way analysis of variance for multiple group comparison) followed by the Dunn's Multiple Comparison test was performed using GraphPad Prism (La Jolla, CA).

The recessive R172P mutation retains the ability to suppress HR in vivo

P53 is a potent tumor suppressor and plays an important role in protecting the genome from endogenous and exogenous damage. HR is the only DNA repair mechanism that is able to mend any lesion with high fidelity when it is working correctly. P53, although not a direct player in HR, helps the cell decide whether HR will be the best route to take. The R172P point mutation in p53 results in a mouse that is unable to transcribe apoptotic genes but is still able to arrest the cell cycle and retains most of its protein: protein interactions.

The frequency of p^{un} reversion was determined in *p53*$^{R172P/R172P}$ $p^{un/un}$ mice using the *in vivo* p^{un} assay (Table 1 and Figure 1). There was no significant increase in the number of eyespots compared to wild type. Suggesting that p53-R172P mutant mice retain the ability to suppress homologous recombination similar to wild type mice. The inability to transcribe apoptotic genes in this particular mutant had no impact on its ability to suppress HR, suggesting that the mechanism for p53 involvement in HR may be cell cycle mediated or through protein: protein interactions.

The aggressive R172H mutant mice show increased HR frequency similar to p53 null mice in vivo

The R172H point mutation results in a protein being formed but it is unable to bind and transcribe any of the p53 target genes. Also many of the normal protein: protein interactions have also been

disrupted. The p53- R172H mutation is more detrimental than the R172P mutation in that the former mice have an earlier onset of tumors and a higher incidence of metastatic tumors in the heterozygous genotype.

The frequency of p^{un} reversion events in the p53-R172H mouse was significantly higher than the wild type controls and p53-R172P mutant mice ($p < .001$) (Table 1 and figure 1). Interestingly, the p^{un} reversion frequency was similar to that of a p53 null animal that produces no p53 protein at all (~ 10 eyespots).

Table 1. Summary of RPE analyzed and p^{un} reversion frequency by p53 genotype

	TOTAL				AVERAGE					
Genotype	RPE	Eyespots	Cells		Eyespots per RPE	SD	Cells per RPE	SD	Spot Size	SD
$p53^{+/+}$ $p^{un/un}$	41	153	441		3.7	2.3	10.7	11	2.8	3.5
$p53^{neo/neo}$ $p^{un/un}$	22	258	680		11.7	6.6	31	26.6	2.6	3.4
$p53^{R172P/R172P}$ $p^{un/un}$	29	118	323		4.1	2.6	11.5	15.4	2.7	5.2
$p53^{R172H/R172H}$ $p^{un/un}$	35	340	742		9.7	4.6	21.2	13.9	2.2	2.5

Table 1: Summary of Total RPE collected and analyzed.

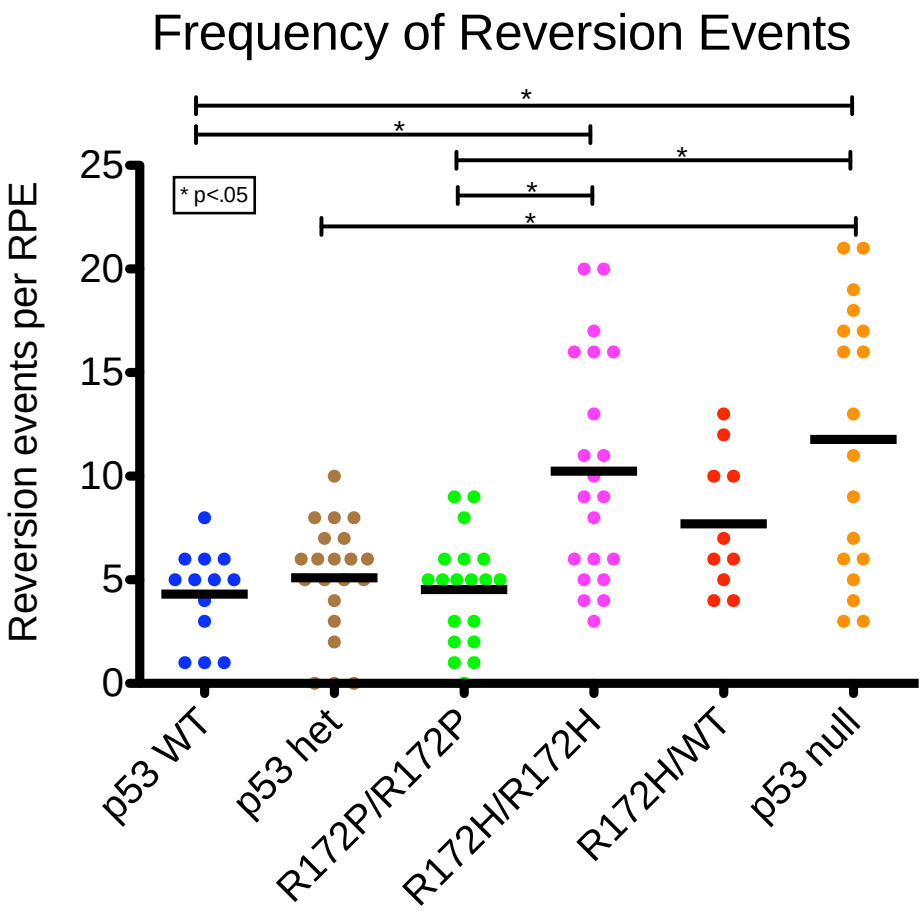

Figure 1. The frequency of eyespots was determined using the pun assay. The data indicates that p53 R172P mutant mice retained the ability to suppress HR similar to WT p53 mice. However, p53 R172H mutant mice showed increased HR frequency similar to p53 null mice. $p<0.001$.

A more sophisticated measurement that can be made using the pun eye assay is the relative position of the eyespot on the RPE. We performed this analysis (Figure 2.) and were surprised to see that there was a difference in these results in comparison to previous findings by the PI's mentor. Previous work showed that p53 null animals showed an increased number of eyespots closer to the optic nerve (towards the center) indicating a time in early development ~E8. This was not seen in the current work and can be explained by the difference in where the cutoff is made in the edge of the RPE. The current work shows a larger portion of the RPE whereas the previous work was a tighter circle around the RPE. This would lead to more spots being counted on the edge of the RPE that what was previously reported (14). There is no significant difference between the p53 mutants and WT in terms of their positional distribution on the RPE. Thus these events are not time in development as was previously reported for p53 null mice (14).

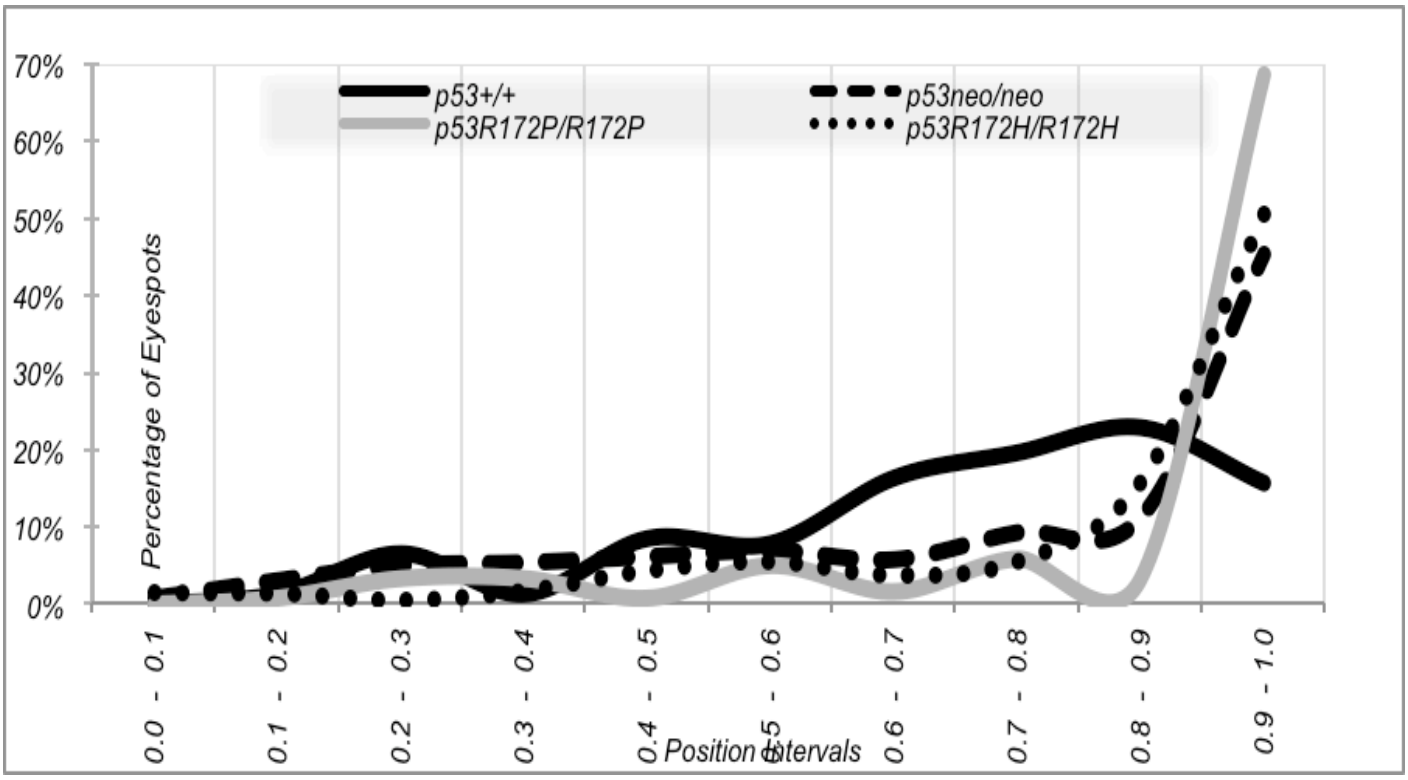

Figure 2. The relative position distribution of eyespots was determined using the pun assay. P<0.05

Because these mouse models are not a true separation of function (none exist in mouse models) we needed to further explore the relationship between p53's transcription factor capability and protein: protein interaction capability to delineate which have been disrupted between the R172P and R172H mutant to cause the change in HR suppression.

The microarray analysis showed few relevant genes that were different between R172P and R172H p53 mutants

We performed microarray analysis to determine transactivation differences between R172P and R172H mutant mice. We used an Agilent whole mouse array on mouse embryonic fibroblasts from each p53 mutant. We focused our analysis on homologous recombination, cell cycle, and apoptotic genes that may be dysregulated between the two mutants (Figure 3).

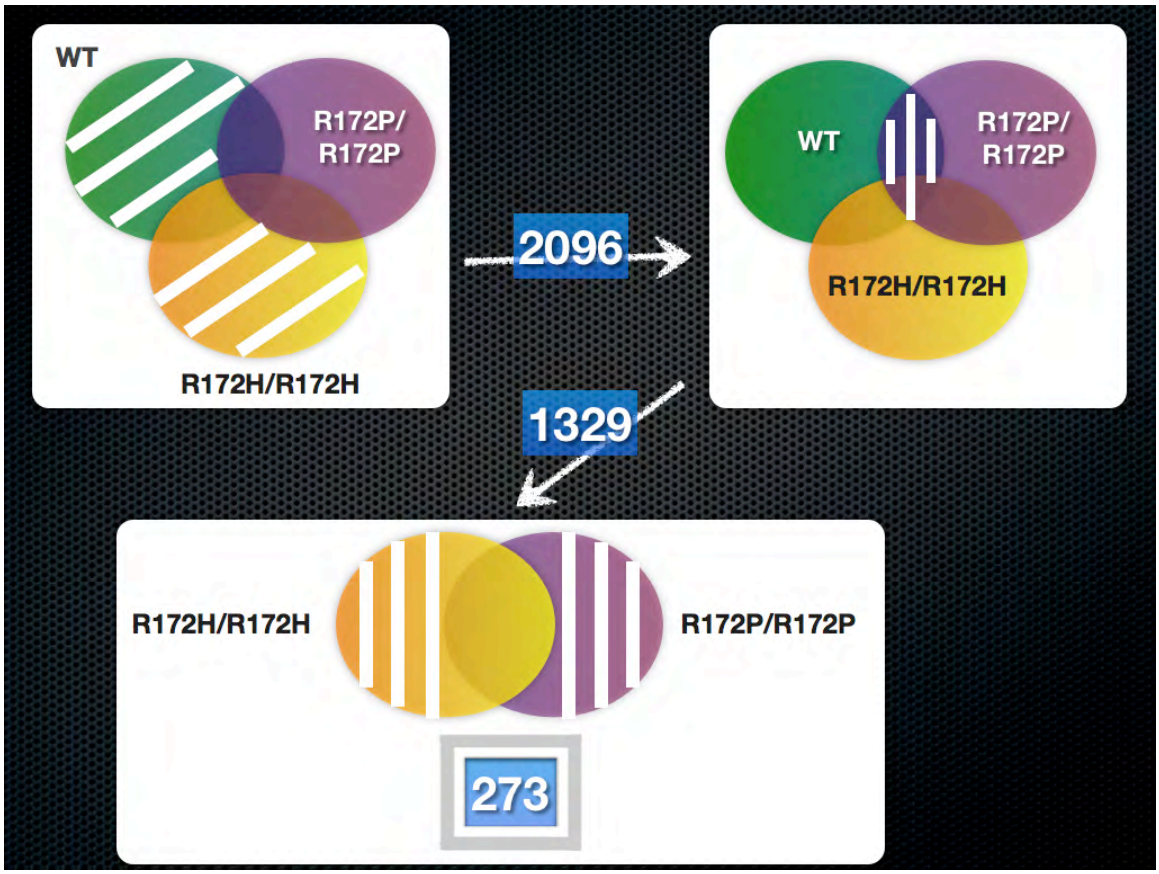

Figure 3: Schematic of the strategy used to narrow down the gene hit list. We compared 2 p53 mutants with WT resulting in 2096 genes. This comparison was then further narrowed by taking out all those genes that overlapped between WT and R172P (HR frequency was not different between these genotypes) resulting in a final gene list of 273 genes that were different between R172H and R172P.

We did not find any relevant genes that were differentially expressed between the two mutants. We compared our hit list of 273 genes to known p53 target genes and found no overlapping genes (Appendix 1). We thus attempted to see if there were indirect genes that may be in common with our hit list and known p53 target genes. Using an in-house analysis software created by Mark Doderer from the GCCRI bioinformatics core we narrowed our list further to 9 genes that might be relevant to the difference in homologous recombination we see between the p53 mutants (Figure 4). Of these 9 genes two are relevant in HR repair- Rad52 and XRCC3 (codes for rad51 protein). We will next validate these two hits in CO-IP and RT-PCR experiments. We are currently optimizing Rad51 and Rad52 antibodies in western blot and have attempted several rounds of Coimmunoprecipitation experiments with little success. We will attempt to tag these proteins with FLAG or HA in order to

have enough protein pulled down to determine if an interaction is broken between mutant p53 and rad51/52. The problem is that p53 is expressed in very low levels in an undamaged cell therefore tagging p53 or over expressing p53 will allow us to detect the p53 protein and bound proteins.

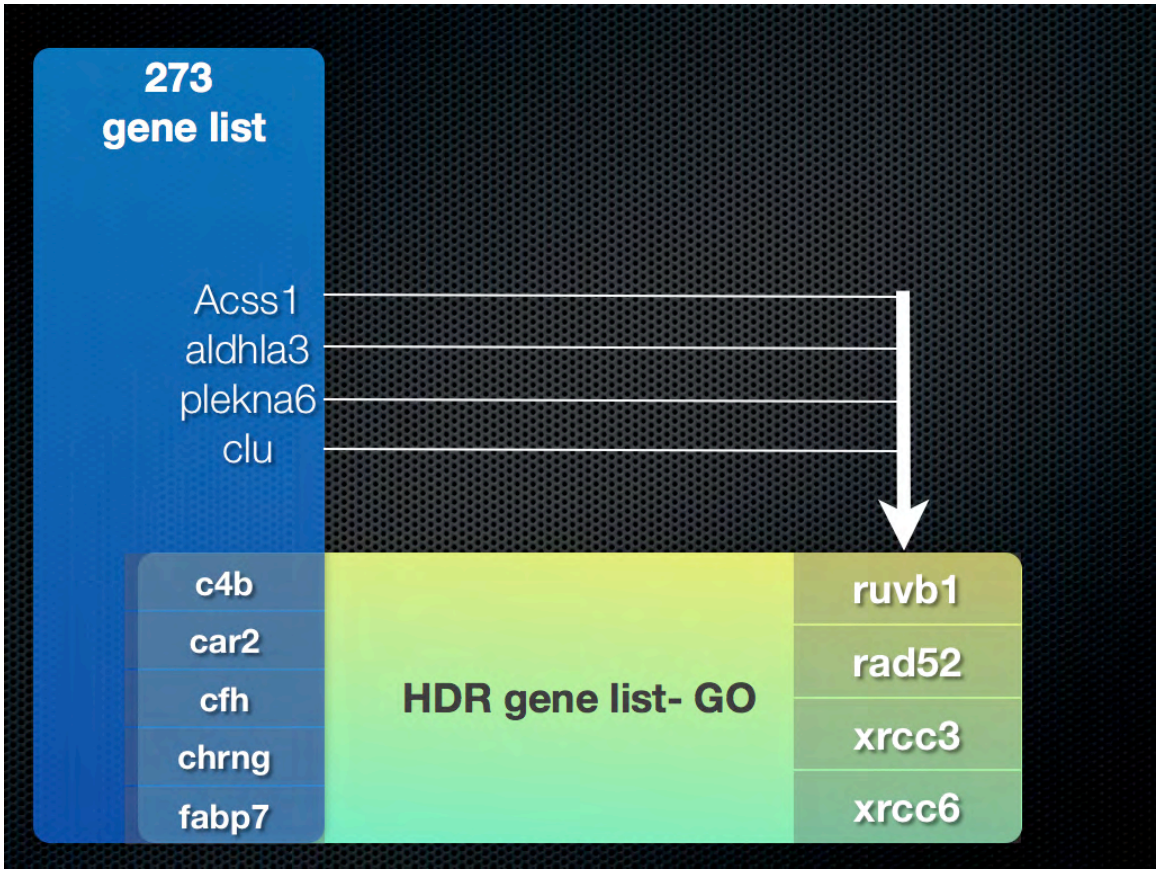

Figure 4: Using Sidekick (created by Mark Doderer) we compared our gene list of 273 genes with homologous directed repair genes (based on Gene Ontology). This comparison resulted in 9 genes.

Protein-Protein interaction analysis proves to be difficult in undamaged cells due to very low p53 protein expression.

To determine if a protein: protein interactions are disrupted in p53 mutant cells causing the loss of suppression of HR we attempted to perform co-immunoprecipitation analysis on WT, R172p, R172H and neo MEFs. We focused on proteins that were relevant in HR such as BRCA/2, Rad51, RPA, 53bp1 and bcl2 to determine if there are broken interactions in the R172H mutation and not in the R172P mutant. We have come across the problem of too little p53 protein being pulled down to determine an interaction or lack of one. P53 is expressed in very low levels in the cell when it is in a

spontaneous undamaged state. In order to have enough protein pull down to determine interactions we will need to damage the cells (IR) or over express and tag p53 (FLAG or HA etc.). We are currently working to optimize these conditions.

Sub-cellular mislocalization of HR proteins might be what is dysfunctional in the R172H mutant causing lack of suppression of

Another scenario that might be occurring in the R172H mutant causing the dysregulation of HR is a mislocalization of HR machinery. In order for the cell to execute efficient HR, the main players such as Rad51, Brca1 etc. must be in the nucleus. In our preliminary Rad51 foci studies we see a cloudy staining of rad51 in the cytoplasm in the p53 mutant MEFs instead of distinct nuclear foci, which is indicative of efficient repair. We thus performed sub-cellular protein fractionation in our samples to determine if the HR proteins are mislocalized in a compartment of the cell where they are not able to execute their function. We currently have nuclear, cytoplasmic, and chromatin-bound lysates and are in the process of evaluating for rad51, rad52, brca1, 53bp1 and other HR proteins.

Specific Aim 2: Determine the influence of the two p53 mutant mice on BER and NER activity as a mechanism by which p53 suppresses homologous recombination.

The NER and BER assays are currently being optimized in the lab. All resources and relevant p53 mutant cells have been collected. This aim will be the focus in the third year.

Specific Aim 3. Determine whether either of the p53 mutants can alter the damage induced HRR response and if their reduced functionality impacts damage induced BER and NER activity.

In the first year of the grant sufficient mice were generated for in vivo pun analysis for spontaneous and damage induced experiments. In the second year of this grant we performed timed matings to intercross heterozygous mice in each cohort $p53^{R172P/+}$ $p^{un/un}$, $p53^{R172H/+}$ $p^{un/un}$ and $p53^{neo/+}$ $p^{un/un}$. The pregnant dams were then exposed to 1Gy of X-ray at E12.5 (Table 2). We are in the process of weaning the pups from exposed dams and harvesting their eyes to assess the frequency of damage induced p^{un} HRR deletion for each *p53* genotype by performing the p^{un} eyespot assay.

Genotype	Dams irradiated	Dams needed
R172P	3	10
R172H	8	10
Neo	9	10

Table 2: Table of plugged female mice that have been irradiated at day 12.5. Eyes are harvested from pups born at day P30.

Key Research Accomplishments

- R172P mutant mice are able to suppress HR similar to wild type suggesting the mechanism is not due to the transactivation of apoptotic genes but through cell cycle or protein: protein interactions.
- R172H mutant mice have a decrease in HR similar to p53 null mice, which do not produce p53 protein. This suggests a protein: protein interaction defect and a possible indirect regulatory role for p53 in the regulation of HR.

Reportable outcomes:

Peer-Reviewed Publications:

- Ravi D, Chen Y, **Karia B**, Brown A, Gu TT, Li J, Carey MS, Hennessy BT, Bishop AJ (Jan 2011). 14-3-3 sigma expression effects G2/M response to oxygen and correlates with ovarian cancer metastasis. *PLoS One*, 6(1):e15864.

- Lin S, Yu L, Yang J, Liu Z, **Karia B**, Bishop AJ, Jackson J, Lozano G, copland JA, Mu X, Sun B, Sun LZ (Dec 2011) Mutant p53 disrupts role of ShcA protein in balancing Smad protein-dependent and independent signaling activity of transforming growth factor-B (TGF-B). *J Biol Chem. 286(51):44023 34.*

- Zimmer SN, Lemieux ME, **Karia BP**, Day C, Zhou T, Zhou Q, Kung AL, Suresh U, Chen Y, Kinney MC, Bishop AJ, Rebel VI (Dec 2011). Mice heterozygous for CREB binding protein are hypersensitive to y-radiation and invariably develop myelodysplastic/myeloproliferative neoplasm. *Exp Hematol. Epub ahead of print.*

Conference/Poster Presentations:

- **Bijal Karia**, Alexander J.R. Bishop. P53 Suppression of Homologous Recombination and Tumorigenesis. Keystone Symposia: Genomic Instability and DNA Repair. Keystone, Colorado. January 30 - February 4, 2011

- **Bijal Karia**, Alexander J.R. Bishop. P53 Suppression of Homologous Recombination and Tumorigenesis. Era of Hope Conference. Orlando, Florida.
August 2-5, 2011

Conclusions

The main focus of this grant was to train me for future as an independent breast cancer investigator. Using the funds from this grant this year I have attended 2 meetings related to genomic instability (Keystone Symposia), as well as the Era of Hope Meeting where I interacted with fellow DoD awardees. My attendance at these meetings allowed me to make contacts with breast cancer investigators all over the world. I was exposed to cutting-edge research that was being done in the field of breast cancer research. My poster presentation allowed for good discussion and feedback from other investigators that will help shape future experiments and thinking about breast cancer.

I have had 1 dissertation committee meeting this year (2-21-2011) in which the discussion of my progress was key. My committee gave me invaluable advice on analysis of experiments, interpretations, statistical help and time management for progression of my PHD.

I have also attended seminars twice a week to better keep up with ongoing research in many fields. This has been a great lesson in critically thinking about the work of others and how they answered questions and solved problems.

I continually meet with my mentors on a weekly basis to discuss experiment results, future experiment planning and troubleshooting strategies.

In this first year I have made significant progress. Animal models are very difficult but I have managed to learn and master mouse husbandry and now have a thriving healthy breeding colony. The cohorts mentioned in the statement of work have all been established and experiments are underway. Assays for measuring RAD51 foci, NER, BER have been learned by the PI and are currently being used in the lab.

The most significant finding that has come from the second year of this grant is that there is a difference between the two p53 mutants in terms of homologous recombination frequency. Given the separation of function of these two mutants we can now tease out the mechanism for how p53 suppresses homologous recombination both in a spontaneous situation and following damage. The microarray analysis showed no differential expression of HR relevant genes between the mutants as and thus our focus will be on determine what protein-protein interaction has been disrupted between the mutants that might explain the difference in HR frequency. We are currently optimizing these experiments and hope to submit a manuscript on these findings in March 2012.

The knowledge and expertise that I have gained and the mouse models that have been established have led to 2 other publications in the second year of this grant that relate to p53 mutation and DNA repair.

“So what”

the significance of these initial findings is that we are closer to determining what p53 mutations are exactly doing and not doing in cells. If we can determine what main “normal” functions of p53 are altered or lost or broken in cancer cells we can develop better targets and therapies that address these issue particularly. For example if it is determined that the more aggressive R172H mutation has a broken protein: protein interaction that causes it to have hyper recombination leading to genomic instability leading to cancer than there is a chance for targeted therapy to repair this interaction in order to restore normal DNA repair function. The research that has been done in this field by previous investigators has been on in vitro plasmid based models with questionable results. Here we use an in vivo assay in a clean genetic system that provides an excellent model for determining genomic instability by way of measuring HR.

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Appendix-1

microarray hit list	known p53 target genes			
273 genes (yy22)	known p53 target genes	matches	FC_HH_PP	direction in HH
Cdkn2a	**Ccnb1**	10	4.409	upregulated
Cdkn2a	**Ccng1**	10	4.888	upregulated
Cdkn2a	**Ccng1**	10	4.773	upregulated
Cdkn2a	**Cd82**	10	4.453	upregulated
Cdkn2a	**Cdc25c**	10	4.440	upregulated
Cdkn2a	**Cdc25c**	10	4.821	upregulated
Cdkn2a	**Cdc2a**	10	4.840	upregulated
Cdkn2a	**Cdc2a**	10	4.428	upregulated
Cdkn2a	**Cdk4**	10	4.722	upregulated
Cdkn2a	**Cdkn1a**	10	4.603	upregulated
Mmp13	**Fos**	1	-6.912	downregulated
Perp	**Il6**	1	-47.175	downregulated
Pgam2	**Il6**	1	-3.594	downregulated
Serpinb5	**Mdm2**	1	-22.723	downregulated
Srgn	**Mrpl41**	1	-12.622	downregulated
1190003J15Rik	1190002H23Rik	0	-8.434	downregulated
2010005H15Rik	Abcb1b	0	-10.230	downregulated
2210011C24Rik	Abcb1b	0	-4.580	downregulated
2210409E12Rik	Abcb1b	0	-9.085	downregulated
2210409E12Rik	Acta2	0	-4.603	downregulated
2310043J07Rik	Acta2	0	-7.862	downregulated
2810432L12Rik	Acta2	0	-2.901	downregulated
4933413A10Rik	Acta2	0	-5.923	downregulated
5730410E15Rik	Acta2	0	-3.963	downregulated

6330403K07Rik	Acta2	0	-7.923	downregulated
6330530A05Rik	Acta2	0	-2.944	downregulated
9130213B05Rik	Acta2	0	-3.636	downregulated
9930013L23Rik	Acta2	0	-9.753	downregulated
9930013L23Rik	Acta2	0	-9.045	downregulated
9930013L23Rik	Acta2	0	-6.153	downregulated
A_52_P1004880	Acta2	0	-6.113	downregulated
A930038C07Rik	Acta2	0	-15.082	downregulated
Aard	Acta2	0	-8.707	downregulated
Ablim3	Afp	0	-5.628	downregulated
Acss1	Afp	0	-24.660	downregulated
Actn2	Afp	0	-12.365	downregulated
Adora1	Afp	0	-11.359	downregulated
AK086961	Afp	0	-16.885	downregulated
Akap6	Afp	0	-5.967	downregulated
Akp2	Afp	0	-5.328	downregulated
Aldh1a3	Afp	0	-3.611	downregulated
Alms1	Afp	0	2.864	upregulated
Apod	Afp	0	-3.415	downregulated
Apoe	Apaf1	0	-9.794	downregulated
Apoe	Apaf1	0	-9.220	downregulated
Apoe	Apaf1	0	-9.651	downregulated
Apoe	Bai1	0	-9.603	downregulated
Apoe	Bai1	0	-9.827	downregulated
Apoe	Bax	0	-9.979	downregulated
Apoe	Bax	0	-9.860	downregulated
Apoe	Bax	0	-10.074	downregulated
Apoe	Bax	0	-9.727	downregulated

Apoe	Bax	0	-9.720	downregulated
Atp1a2	Bax	0	-18.437	downregulated
BC099439	Bax	0	-29.442	downregulated
BC117090	Bax	0	-30.091	downregulated
BC117090	Bax	0	-33.872	downregulated
BC117090	Bax	0	-30.050	downregulated
BU920841	Bbc3	0	-5.938	downregulated
BY439412	Bcl2	0	-8.238	downregulated
C1qa	Bcl2	0	-8.371	downregulated
C1qc	Bcl2	0	-5.394	downregulated
C3	Bcl2	0	-16.408	downregulated
C3	Bcl2	0	-16.677	downregulated
C3	Bcl2	0	-15.996	downregulated
C3	Bcl2	0	-15.358	downregulated
C3	Bcl2	0	-16.662	downregulated
C3	Bcl2	0	-16.520	downregulated
C3	Bcl2	0	-17.032	downregulated
C3	Bcl2	0	-16.546	downregulated
C3	Bcl2	0	-17.907	downregulated
C3	Bdkrb2	0	-17.259	downregulated
C4b	Birc5	0	-7.503	downregulated
Cacna1s	Brca1	0	-8.795	downregulated
Car2	Brca1	0	-8.964	downregulated
Casq2	Btg2	0	-10.013	downregulated
Cav3	Btg2	0	-6.843	downregulated
Cbr1	Btg2	0	-5.437	downregulated
Ccdc116	Casp1	0	3.498	upregulated
Ccl5	Casp6	0	-3.956	downregulated

Cd200	Ccnb1	0	-7.538	downregulated
Cdh13	Ccnb1	0	-3.985	downregulated
Ceacam2	Cdkn1a	0	-5.721	downregulated
Celsr1	Cdkn1a	0	-27.824	downregulated
Cfb	Cdkn1a	0	-9.096	downregulated
Cfh	Cdkn1a	0	-24.207	downregulated
Cfh	Cdkn1a	0	-8.093	downregulated
Chrna1	Cdkn1a	0	-9.927	downregulated
Chrng	Cdkn1a	0	-23.899	downregulated
Clec4d	Cdkn1a	0	-5.624	downregulated
Clu	Cdkn1a	0	-9.058	downregulated
Cma1	Cdkn2a	0	-4.766	downregulated
Cntn1	Cdkn2a	0	-4.710	downregulated
Col22a1	Cdkn2a	0	-3.901	downregulated
Col2a1	Cdkn2a	0	-17.342	downregulated
Col5a3	Cdkn2a	0	-3.302	downregulated
Col9a2	Cdkn2a	0	-3.896	downregulated
Cox8b	Cdkn2a	0	-3.560	downregulated
Cpb1	Cdkn2a	0	3.532	upregulated
Crym	Cdkn2a	0	-5.231	downregulated
Cuedc1	Cdkn2a	0	5.486	upregulated
Cuedc1	Chek1	0	4.265	upregulated
Cxcl14	Chek1	0	-3.756	downregulated
Cxcl16	Ckm	0	-3.144	downregulated
Cxcl4	Ctnnb1	0	-7.022	downregulated
Cyp26b1	Ctnnb1	0	-9.272	downregulated
Cyp51	Ctnnb1	0	3.108	upregulated
D430036J16Rik	Ctnnb1	0	4.430	upregulated

Dct	Ctnnb1	0	-97.213	downregulated
Ddit4l	Ctnnb1	0	-2.959	downregulated
Ddit4l	Ctnnb1	0	-3.368	downregulated
Ddx4	Ctnnb1	0	16.300	upregulated
Dhcr24	Ctnnb1	0	2.877	upregulated
Dio3	Ctnnb1	0	-5.421	downregulated
Dok7	Ctnnb1	0	-10.989	downregulated
Dscr1l1	Ctnnb1	0	-2.867	downregulated
EG433016	Ctsd	0	-11.387	downregulated
Elovl6	Ctsd	0	2.845	upregulated
Elovl7	Cx3cl1	0	-4.512	downregulated
Emid2	Cx3cl1	0	-14.981	downregulated
Enpp2	Dab2ip	0	-18.235	downregulated
Epha3	Ddb1	0	4.163	upregulated
Epyc	Dkk1	0	-29.371	downregulated
Esco1	Dkk1	0	2.814	upregulated
Esco1	Ecm1	0	3.853	upregulated
Expi	Ecm1	0	-8.228	downregulated
Fabp7	Eef1a1	0	-14.370	downregulated
Fmod	Eef1a1	0	-3.587	downregulated
Galntl1	Egfr	0	-3.701	downregulated
Gpr149	Egfr	0	6.196	upregulated
Gprasp2	Egfr	0	-8.921	downregulated
Gprc5c	Egfr	0	-5.598	downregulated
Gprc5c	Egfr	0	-4.395	downregulated
Gvin1	Egfr	0	2.956	upregulated
Gzmd	Egfr	0	-3.437	downregulated
H2-Q10	Egfr	0	-5.190	downregulated

Heyl	Egfr	0	-3.268	downregulated
Hist2h2bb	Egfr	0	5.161	upregulated
Hoxc5	Egfr	0	7.113	upregulated
Hoxc6	Egfr	0	3.825	upregulated
Hsd11b1	Egfr	0	-9.275	downregulated
Ica1	Egfr	0	3.844	upregulated
Id4	Egfr	0	-3.858	downregulated
Id4	Egfr	0	-2.956	downregulated
Idi1	Egfr	0	4.372	upregulated
Igf2	Egfr	0	-3.069	downregulated
Igf2	Egfr	0	-3.246	downregulated
Igf2	Egfr	0	-3.186	downregulated
Igf2	Egfr	0	-3.173	downregulated
Igf2	Egfr	0	-3.342	downregulated
Igf2	Egfr	0	-3.232	downregulated
Igf2	Egfr	0	-3.194	downregulated
Igf2	Ei24	0	-3.277	downregulated
Igf2	Fas	0	-3.153	downregulated
Igf2	Fas	0	-3.258	downregulated
Igf2	Fas	0	-3.477	downregulated
Igfbp2	Fas	0	-14.006	downregulated
Il31ra	Fas	0	7.839	upregulated
Itga4	Fas	0	4.272	upregulated
Itgav	Fas	0	3.047	upregulated
Itm2a	Fas	0	-3.216	downregulated
Krt16	Fas	0	-10.636	downregulated
Krt17	Fas	0	-6.131	downregulated
Krt5	Fas	0	-4.171	downregulated

Lcn2	Fos	0	-14.604	downregulated
Lmod3	Fos	0	-5.392	downregulated
Loxl3	Fos	0	3.522	upregulated
Lrrc33	Fos	0	-3.604	downregulated
Lrrn1	Fos	0	-8.435	downregulated
Lss	Fos	0	3.224	upregulated
Ltf	Fos	0	-12.736	downregulated
Mcpt4	Fos	0	-4.108	downregulated
Megf10	Fos	0	3.241	upregulated
Mmp3	Gadd45a	0	-78.547	downregulated
Mmp9	Gadd45b	0	-13.325	downregulated
Mmp9	Gadd45b	0	-9.667	downregulated
Mmp9	Gadd45g	0	-16.024	downregulated
Mmp9	Gdf15	0	-22.648	downregulated
Mmp9	Glipr2	0	-13.787	downregulated
Mmp9	Gml	0	-9.640	downregulated
Mmp9	Gml	0	-12.186	downregulated
Mmp9	Gpx1	0	-13.836	downregulated
Mmp9	Gpx2	0	-12.735	downregulated
Mmp9	Gpx3	0	-10.662	downregulated
Mpeg1	Gpx4	0	-3.573	downregulated
Mybph	Gpx5	0	-12.218	downregulated
Myh1	Gpx6	0	-12.705	downregulated
Myh2	Gpx7	0	-6.800	downregulated
Myh3	Gtse1	0	-17.364	downregulated
Myh3	Hbegf	0	-13.812	downregulated
Myh7	Hgf	0	-10.491	downregulated
Myh7	Hgf	0	-12.840	downregulated

Myh8	Hgf	0	-8.518	downregulated
Myl1	Hgf	0	-7.346	downregulated
Mylpf	Hic1	0	-7.148	downregulated
Myo18b	Hspa2	0	-11.816	downregulated
Myo1g	Hspa2	0	-4.548	downregulated
Myod1	Igfbp3	0	-3.889	downregulated
Myod1	Igfbp3	0	-10.346	downregulated
Myod1	Igfbp3	0	-6.056	downregulated
Myod1	Il2	0	-6.417	downregulated
Myog	Il2	0	-7.371	downregulated
Myog	Il2	0	-7.653	downregulated
Myog	Il2	0	-7.162	downregulated
Myog	Il2	0	-9.198	downregulated
Myog	Il2	0	-8.294	downregulated
Myog	Il2	0	-7.614	downregulated
Myog	Il2	0	-7.279	downregulated
Myog	Il2	0	-8.824	downregulated
Myog	Il2	0	-6.874	downregulated
Myog	Il2	0	-6.733	downregulated
Myom2	Il4	0	-6.573	downregulated
NAP102441-1	Il4	0	2.969	upregulated
NAP102683-1	Il4	0	3.033	upregulated
NAP124154-1	Il4	0	6.772	upregulated
Ncf2	Il4	0	-3.284	downregulated
Npr3	Il4	0	-4.895	downregulated
Npy	Il4	0	-7.725	downregulated
Pcsk9	Il4	0	5.072	upregulated
Pdlim3	Il4	0	-3.809	downregulated

Pdlim3	Il4	0	-4.742	downregulated
Pkp1	Il6	0	-16.815	downregulated
Plcl1	Il6	0	5.463	upregulated
Plekha6	Il6	0	-3.501	downregulated
Plxna4	Il6	0	2.969	upregulated
Prg4	Il6	0	-34.291	downregulated
Prkg2	Il6	0	4.053	upregulated
Prnd	Il6	0	-5.292	downregulated
Prss12	Il6	0	-3.582	downregulated
Prss35	Insr	0	-4.751	downregulated
Ptn	Krt8	0	6.778	upregulated
Ptx3	Krt8	0	-3.730	downregulated
Ptx3	Lrdd	0	-3.334	downregulated
Rarres2	Lrdd	0	-20.800	downregulated
Rarres2	Lrdd	0	-18.322	downregulated
Rtn1	Mdm2	0	-6.389	downregulated
S100a8	Mdm2	0	-19.926	downregulated
S100a9	Mdm2	0	-76.564	downregulated
Saa1	Mdm2	0	-8.292	downregulated
Saa3	Mdm2	0	-21.669	downregulated
Scn3b	Mdm2	0	-10.730	downregulated
Scn5a	Mdm2	0	-8.937	downregulated
Scube1	Mdm2	0	-7.061	downregulated
Serping1	Mdm2	0	-4.767	downregulated
Slc16a6	Mdm2	0	-2.974	downregulated
Slpi	Met	0	-11.207	downregulated
Snx24	Met	0	2.998	upregulated
Snx24	Met	0	4.197	upregulated

Sorbs2	Mmp13	0	-3.926	downregulated
Sparcl1	Mmp1a	0	-29.972	downregulated
Sparcl1	Mmp1a	0	-31.026	downregulated
Spon1	Mmp2	0	-15.063	downregulated
St6galnac2	Mrpl41	0	-2.828	downregulated
Stard4	Mtap4	0	3.218	upregulated
TC1651696	Mtap4	0	-4.156	downregulated
Thy1	Mtap4	0	2.995	upregulated
Tmem182	Mtap4	0	-7.618	downregulated
Tmem30b	Mtap4	0	-3.751	downregulated
Tnni1	Myc	0	-11.236	downregulated
Tnni1	Myc	0	-7.908	downregulated
Tnnt1	Myc	0	-4.720	downregulated
Trem2	Myc	0	-4.088	downregulated
Trf	Myc	0	-4.752	downregulated
Trim63	Myc	0	-4.120	downregulated
U90926	Myc	0	-9.384	downregulated
Unc13a	Myc	0	3.168	upregulated
Unc5b	Myc	0	3.386	upregulated
Vcan	Myc	0	3.371	upregulated
Wars2	Myc	0	3.432	upregulated
Wdr40b	Myc	0	-3.155	downregulated
Wnt10a	Ndrg1	0	-12.063	downregulated
Zfp185	Nos3	0	-5.004	downregulated
Zfp318	Nos3	0	-5.097	downregulated
	Nos3			
	Nos3			
	Nos3			

Nos3

Nos3

Nos3

Nos3

Nos3

P2rxl1

P2rxl1

Pcbp4

Pcna

Pcna

Pcna

Pcna

Pcna

Pcna

Pcna

Pcna

Pcna

Pcna

Perp

Pgam2

Plagl1

Plagl1

Pmaip1

Pold1

Pold1

Polk

Polk

Ppm1d

Ppm1d